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Metabolic requirements for release of endogenous noradrenaline during myocardial ischaemia and anoxia

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1 The metabolic conditions required for noradrenaline (NA) release from ischaemic and anoxic perfused hearts of the rat were studied.

2 Forty minutes of flow reduction to approximately $0.25\,\mathrm{ml\,g^{-1}\,min^{-1}}$ did not elicit enhanced noradrenaline overflow from the isolated heart perfused with normoxic perfusate even in the absence of added substrate. Enhanced overflow did occur when substrate-free ischaemia was induced after a 60 min period of substrate-free perfusion.

3 Noradrenaline overflow was enhanced by perfusion at normal flow rates with an anoxic ($P\mathrm{O}_2 < 1$ mmHg) perfusate containing no substrate. Such enhanced overflow occurred in the absence of calcium in the perfusate and was almost completely abolished by the addition of 11 mM glucose.

4 Enhanced noradrenaline overflow occurring either during low flow ischaemia after substrate deprivation or during anoxic substrate-free perfusion at normal flow rates was markedly suppressed by desipramine.

5 Exocytotic noradrenaline overflow induced by electrical stimulation of the left cervico-thoracic ganglion continued unchanged during 60 min of anoxia if the perfusate contained 11 mM glucose. In the absence of added substrate there was a decline in the overflow induced by such stimulation which was more rapid with anoxic than normoxic perfusate.

6 Re-introduction of calcium, oxygen and substrate after 10, 20 or 30 min of calcium-free, substrate-free, anoxic perfusion was associated with a massive overflow of the intracellular enzyme lactate dehydrogenase. At 10 min there was an associated transient minor increase in NA overflow but at 20 and 30 min the overflow of NA, elevated as a result of anoxic perfusion, returned to pre-anoxic levels on the re-introduction of substrate and oxygen.

7 These studies demonstrate a central role for the metabolic status of the sympathetic nerve terminal in determining the magnitude of exocytotic and nerve-impulse independent noradrenaline release from the heart. During the course of myocardial ischaemia *in vivo* nerve-impulse independent release would be expected to occur only in regions of severe flow reduction. This may produce heterogeneous stimulation of the myocardium.

Introduction

Catecholamine release within the heart is thought to play an important role in modulating the course of myocardial ischaemia, particularly with regard to the development of serious arrhythmias. Catecholamines could be released within the heart following reflex neural activity in the cardiac sympathetic nerves and enhanced sympathetic activity has been detected during early myocardial ischaemia (Malliani *et al.*, 1969). However, early in severe ischaemia there is a depression of the noradrenaline release produced by stimulation of cardiac sympathetic nerves (Dart *et al.*, 1984). Catecholamine release may also occur independently of reflex activity since catecholamine overflow has been found to occur from isolated perfused hearts made ischaemic (Schömig *et al.*, 1984; Abrahamsson *et al.*, 1985). The mechanism of this release is different from neurally mediated exocytotic release and its behaviour is compatible with a carrier-mediated efflux (Paton, 1976; Schömig *et al.*, 1984). The aim of the present studies was to define more precisely the

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metabolic conditions necessary for nerve-impulse independent release to occur. In order to distinguish reperfusion from ischaemic processes, a distinction not possible in earlier studies (Schömig *et al.*, 1984), experimental models not dependent on total ischaemia with subsequent reperfusion have been used. Furthermore, anoxia at unchanged flow rate has also been used to study the effect of changes in oxygen and substrate supply in the absence of the secondary effects of low flow ischaemia: changes in external ion concentration, acidosis and metabolite accumulation. Study of anoxia at unchanged flow also circumvents the difficulty that, under conditions of low flow, neuronal reuptake of exocytotically released noradrenaline (NA) becomes an increasingly important clearance mechanism (Dart & Riemersma, 1985). The effects of subsequent re-introduction of oxygen and substrate were studied, in combination with the re-introduction of calcium, to determine whether the sympathetic nerve terminals underwent damage analogous to the calcium/oxygen paradox (Hearse *et al.*, 1978). In complementary experiments the metabolic requirements of release in response to nerve stimulation were also studied.

These studies have been presented, in part, at the Winter Meeting (1985) of the British Pharmacological Society.

Methods

All experiments were performed on male Wistar rats (150–200 g) anaesthetized with thiopentone (50 mg kg^{-1} i.p).

Non-innervated hearts

Following anaesthesia heparin (500 u) was injected into the inferior vena cava. The thorax was then opened and the heart excised and rapidly mounted for perfusion by the Langendorff method. All experiments were performed with constant flow perfusion using a Watson Marlow multichannel peristaltic pump (202U). In all experiments an initial perfusion with a modified Krebs-Henseleit buffer (composition (mM): Na^+ 141.5, K^+ 4.05, Ca^{2+} 1.85, Mg^{2+} 1.05, Cl^- 128.0, HCO_3^- 25.0, PO_4^{3-} 0.38, EDTA 0.0269) containing 5.5 mM glucose and 1.8 mM pyruvate was performed for at least 20 min before any intervention. Hearts were mounted in chambers (4–8 simultaneously) kept at 37.5°C and perfusate was 37.5°C at the point of entry into the heart and had a pH of 7.4 (produced by gassing with 95% O_2 plus 5% CO_2). P_{O_2} was >500 mmHg. Hearts were weighed at the end of each experiment.

Ischaemic experiments

Ischaemia was induced by reducing the flow rate from 5.50 ± 0.15 ml g^{-1} min^{-1} to 0.246 ± 0.006 ml g^{-1} min^{-1}. The following experiments were performed: (1) Ischaemia with perfusate containing 5.5 mM glucose and 1.8 mM pyruvate ($n = 11$). (2) As (1), but with 100 nM desipramine (Ciba-Geigy) begun 20 min before the ischaemic episode and continued throughout the remainder of the experiment ($n = 11$). (3) Ischaemia with perfusate containing no substrate (substrate-free series, $n = 7$). (4) Substrate depletion was induced by a 60 min substrate-free, normoxic, perfusion. Ischaemia, also substrate-free, was then induced (substrate-depleted series, $n = 8$). (5) As (4) but with 100 nM desipramine begun 20 min before the ischaemic period and continued for the remainder of the experiment ($n = 8$).

Anoxia experiments

Anoxic experiments were all performed at unchanged flow rates (4.88 ± 0.15 ml g^{-1} min^{-1}). Anoxic perfusate was produced by gassing the perfusate with 95% N_2 and 5% CO_2. Remaining traces of oxygen were removed by the addition of the reducing agent sodium dithionite (final concentration 0.5 mM). Anoxia (P_{O_2}<1 mmHg) was confirmed by P_{O_2} measurement (Instrumentation Laboratory System 1302 pH/blood gas analyser).

The following experiments were performed: (1) Perfusion with anoxic perfusate containing no substrate ($n = 15$). (2) Perfusion with anoxic perfusate containing no substrate and no calcium ($n = 7$). (3) Substrate depletion was induced by 60 min perfusion with a normoxic perfusate containing no substrate. Anoxic perfusion, also without substrate, was then instituted ($n = 7$). (4) Perfusion with an anoxic perfusate containing 11 mM glucose ($n = 7$). (5) As (1) but with the perfusate containing 100 nM desipramine, started 20 min before anoxia and continued for the remainder of the experiment ($n = 8$).

In all experiments (ischaemia and anoxia) investigating the action of desipramine an equal number of control and desipramine treated hearts were perfused simultaneously.

Re-oxygenation experiments

In these experiments re-oxygenation was combined with re-introduction of calcium in order to determine whether the calcium/oxygen paradox (Hearse *et al.*, 1978) would cause noradrenaline release from the nerve terminals, or whether the re-introduction of oxygen and substrate would allow rapid restoration of nerve terminal function. In these experiments a low pH was used during anoxia to simulate more closely an 'ischaemic' environment. Flow rates were unchanged ($4.69 \pm 0.11\,ml\,g^{-1}\,min^{-1}$) throughout the experiment. A period of 10, 20 or 30 min (n = 7 for each group) perfusion with an anoxic perfusate containing no substrate or calcium and at pH 6.6 (produced by addition of HCl) was followed by re-introduction of normal perfusate (pH 7.4, $Po_2 > 500$ mmHg, Ca^{2+} 1.85 mM, glucose 5.5 mM and pyruvate 1.8 mM). A pH of 6.6 was chosen since during ischaemia with added substrate the pH of the venous effluent at 10 min was 6.60 ± 0.05 (n = 5).

Innervated hearts

Details of this preparation have been given previously (Dart *et al.*, 1983). In these experiments stimulation was performed on each occasion for 30 s at 5 pulses s^{-1}, 0.8 mA. Effluent was collected before and for 60 s after the start of each stimulation. NA overflow is given as overflow in excess of basal, prestimulation, overflow. The sympathetic ganglion and cardiac sympathetic nerves were superfused with oxygenated buffer containing glucose and hearts were superfused with paraffin warmed to 37.5°C. Apex displacement was recorded (Devices M4) for assessment of heart rate increases. To overcome problems caused by atrio-ventricular block during stimulation the post-stimulation heart rates were determined by measuring the peak rates sustained for 10 s or more during the period of stimulation. Anoxia was produced as above. Flow rates were $4.24 \pm 0.12\,ml\,g^{-1}\,min^{-1}$ and were unchanged throughout each experiment.

The following experiments were performed: (1) Control series: perfusion was performed with normoxic perfusate containing 5.5 mM glucose, 1.8 mM pyruvate and stimulation performed at 0, +15, +30, +45 and +60 min (n = 6). (2) Stimulation was performed before and 10 min after change to an anoxic perfusate containing no substrate (n = 6). (3) Stimulation was performed before and after 15, 30, 45 and 60 min perfusion with normoxic perfusate containing no substrate (n = 6). (4) Stimulation was performed before and after 15, 30, 45 and 60 min perfusion with an anoxic perfusate containing 11 mM glucose (n = 6).

In (2)–(4) the initial stimulation was performed during perfusion with normoxic perfusate containing 5.5 mM glucose, 1.8 mM pyruvate.

Biochemical and statistical analyses

Samples for catecholamine estimation were collected on ice and immediately stabilized by 1:1 addition of 0.6 N perchloric acid. Samples were stored at −40°C until assayed, in duplicate, by the radio-enzymatic method of Da Prada & Zürcher (1976). Assay blanks were typically in the range 30–50 c.p.m. with an inter-assay coefficient of variation of 17% whilst 600 fmol standards yielded 800–1000 c.p.m. with a coefficient of variation of 7%. All samples from each heart (up to a maximum of 4 hearts) were determined in the same assay and, in experiments investigating the action of desipramine, samples from equal numbers of treated and untreated hearts were assayed together. Sodium dithionite (0.5 mM) and desipramine (100 nM) were without effect on the noradrenaline assay (blanks and standards). Samples for lactate and lactate dehydrogenase (LDH) estimation were immediately assayed, in duplicate, using an enzymatic method (Boehringer Mannheim) on a Cobas Bio centrifugal analyser. Potassium concentrations in the effluent were measured with a Corning 435 flame photometer. In calculations of biochemical data the means of the duplicate measurements were used.

Statistical analyses between groups were performed using analysis of variance on a Minitab statistical package (CLE.COM Ltd). Significance is assumed if $P < 0.05$. Values given are mean ± s.e.mean.

Results

The effect of reducing flow from approximately $5\,ml\,g^{-1}\,min^{-1}$ to approximately $0.25\,ml\,g^{-1}\,min^{-1}$ on noradrenaline overflow from the non-innervated heart is shown in Figure 1. In the presence of 5.5 mM glucose and 1.8 mM pyruvate such ischaemia did not increase

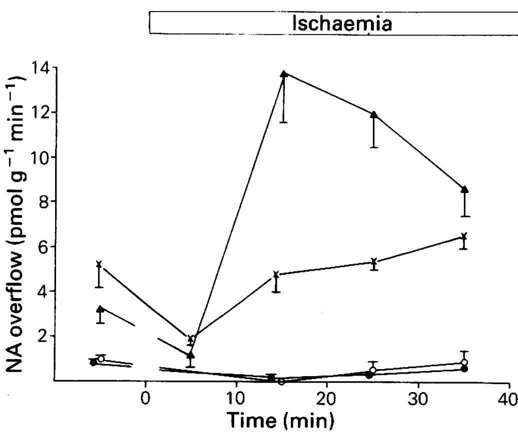

Figure 1 Noradrenaline (NA) overflow ($pmol\,g^{-1}\,min^{-1}$) from hearts made ischaemic for 40 min (open bar) in the presence of 5.5 mM glucose and 1.8 mM pyruvate (○) (n = 11), in the absence of substrate (substrate-free) (●) (n = 7), in the absence of substrate after a 60 min substrate-free pre-perfusion (substrate-depleted) with (×) (n = 8) and without (▲) (n = 8) desipramine (100 nM). Each point represents the mean with vertical lines indicating s.e.mean.

NA overflow with respect to the pre-ischaemic values although during the course of ischaemia there was a significant rise in NA overflow from 0.10 ± 0.02 pmol g^{-1} min^{-1} at 15 min to 1.00 ± 0.42 pmol g^{-1} min^{-1} at 37 min. When ischaemia was produced in the absence of added substrate there was again no increase in NA overflow above pre-ischaemic values but a significant rise during ischaemia from 0.24 ± 0.09 pmol g^{-1} min^{-1} at 15 min to 0.80 ± 0.23 pmol g^{-1} min^{-1} at 37 min. However, when substrate-free ischaemia was produced following a period of substrate depletion there was an enhanced overflow, with respect to pre-ischaemic values, at 15, 25 and 37 but not at 5 min. Cumulative lactate overflows (μmol g^{-1} 40 min^{-1}) during ischaemia were 20.3 ± 2.9 in the substrate-free and 20.5 ± 2.6 in the substrate-depleted series.

Desipramine did not significantly affect the overflow of NA during low flow ischaemia in the presence of glucose and pyruvate (not shown). At 15 min overflows (pmol g^{-1} min^{-1}) were 0.19 ± 0.04 in the desipramine treated series, in comparison to 0.10 ± 0.02 in the control series, whereas at 25 and 37 min desipramine was associated with lower overflows of 0.43 ± 0.11 and 0.60 ± 0.16 in comparison to respective control values of 0.67 ± 0.19 and 1.00 ± 0.42. Pre-ischaemic NA overflow in the desipramine-treated series was 1.19 ± 0.36 pmol g^{-1} min^{-1}. Desipramine was also without effect on lactate overflow during ischaemia. Lactate overflows (μmol g^{-1} min^{-1}) were 0.98 ± 0.16 (15 min), 1.09 ± 0.14 (25 min) and 0.99 ± 0.13 (37 min) in the absence of desipramine, 1.04 ± 0.18, 1.02 ± 0.18 and 0.99 ± 0.19, respectively, in the presence of desipramine. Desipramine, however, did suppress the enhanced NA overflow produced by 60 min substrate depletion followed by substrate-free ischaemia (Figure 1). There was no difference in the control, pre-ischaemic overflows, between the two groups. During such ischaemia, desipramine significantly suppressed the NA overflow at 15 and 25 min but not at 5 and 37 min. Cumulative lactate overflow during substrate-free ischaemia after a period of substrate deprivation was not affected by desipramine at 19.6 ± 3.1 μmol g^{-1} 40 min^{-1}.

Perfusion with an anoxic perfusate containing no substrate resulted in enhanced NA overflow in the presence and absence of extracellular calcium (Figure 2). In the presence of calcium enhanced overflow, in comparison to pre-anoxic values, occurred after 15 (but not 5 or 10) min. However following substrate depletion, induced by 60 min perfusion with normoxic perfusate containing no substrate, substantially increased overflow was already apparent by 10 min. In the absence of calcium, overflow of NA at 20 min (73.90 ± 5.25 pmol g^{-1} min^{-1}) was significantly higher than during normal calcium, substrate-free perfusion (27.0 ± 3.4 pmol g^{-1} min^{-1}). Perfusion

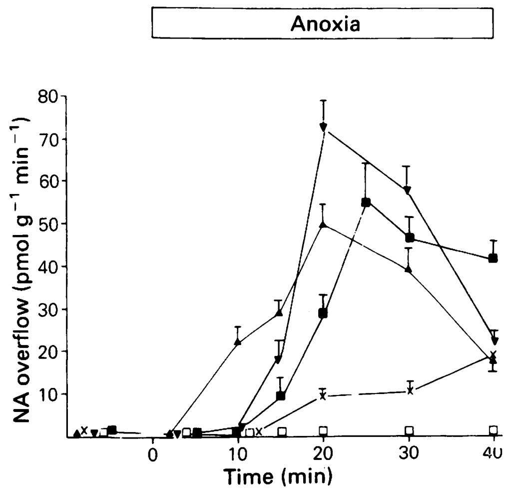

Figure 2 Noradrenaline (NA) overflow (pmol g^{-1} min^{-1}) from hearts made anoxic ($Po_2 < 1$ mmHg) in the presence of 11 mM glucose (□), in the absence of substrate (with (×) and without (■) 100 nM desipramine), in the absence of substrate following a 60 min substrate-free, normoxic, pre-perfusion (▲) and in the absence of substrate and calcium (▼). Flow was unchanged throughout (4.88 ± 0.15 ml g^{-1} min^{-1}). Each point is the mean of 7–15 observations and vertical lines indicate s.e.mean.

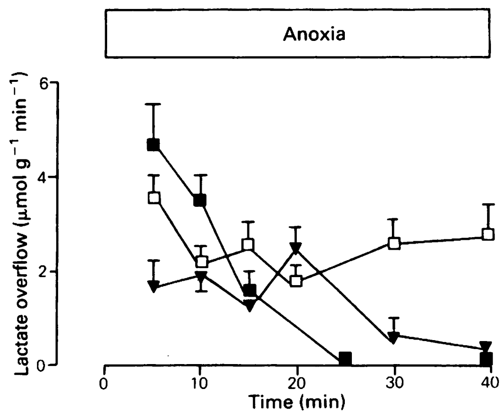

Figure 3 Lactate overflow (μmol g^{-1} min^{-1}) from hearts made anoxic ($Po_2 < 1$ mmHg) in the absence of glucose and calcium (▼), in the absence of glucose (■) and in the presence of 11 mM glucose (□). Flow rates were unchanged throughout. Each point represents the mean ($n = 7$ for each series) and vertical lines indicate s.e.mean.

with an anoxic buffer containing 11 mM glucose almost completely suppressed the enhanced NA overflow (Figure 2). The lactate overflows (μmol g^{-1} min^{-1}) with and without added glucose are shown in Figure 3. In the absence of added substrate but with normal calcium, lactate overflow fell to low levels by 25 min and was undetectable at 40 min. In contrast in the presence of 11 mM glucose, lactate overflow was sustained throughout the experiment. The total lactate overflows during anoxia were 70.8 ± 4.5 μmol g^{-1} 40 min^{-1} in the absence of substrate and 103.7 ± 9.3 μmol g^{-1} 40 min^{-1} in the 11 mM glucose group ($P < 0.02$). Lactate overflow was significantly reduced with a calcium-free perfusate at 52.2 ± 2.9 μmol g^{-1} 40 min^{-1} ($P = 0.0063$ vs glucose free, normal calcium values); following a substrate-free pre-perfusion, lactate overflow was

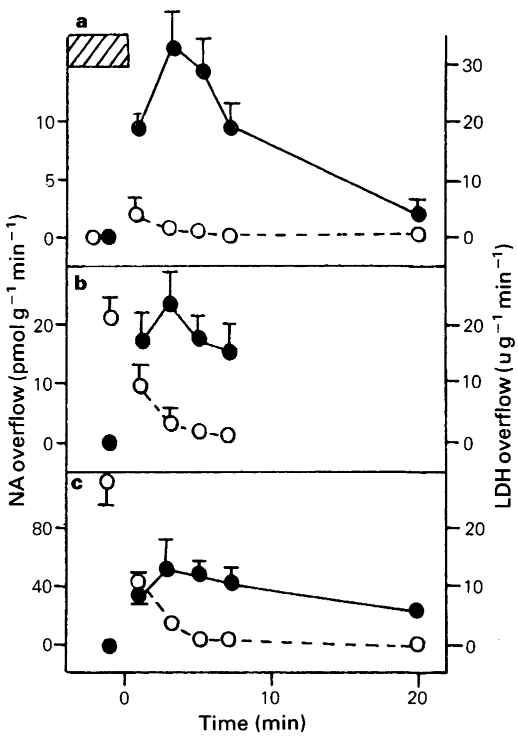

Figure 4 The effect of re-introduction of calcium, oxygen and substrate after 10 (a), 20 (b) and 30 (c) min of calcium-free, substrate-free, acidic (pH 6.6), anoxic perfusion ($n = 7$ for each group) on (O) noradrenaline (NA) overflow (pmol g^{-1} min^{-1}) (note different scales), and (●) lactate dehydrogenase (LDH) overflow (u g^{-1} min^{-1}). Error bars not shown fall within the limits of the symbols. Flow rates were unchanged throughout these experiments (4.69 ± 0.11 ml g^{-1} min^{-1}).

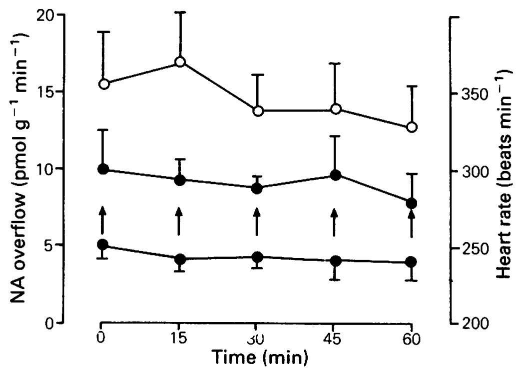

Figure 5 Noradrenaline (NA) overflow (pmol g^{-1} min^{-1}) and heart rate increase produced by repetitive stimulation of the left cervico-thoracic ganglion at 5 pulses s^{-1}, 0.8 mA for 30 s. Perfusate contained 5.5 mM glucose, 1.8 mM pyruvate and had $Po_2 > 500$ mmHg. (O) indicate stimulation-induced NA overflow, (●) indicate heart rate and respective pre- and post-stimulation values are indicated by arrows. Pre-stimulation overflows (pmol g^{-1} min^{-1}) were unchanged with values of 0.75 ± 0.13 (0 min), 1.16 ± 0.24 (15 min), 0.80 ± 0.09 (30 min), 0.66 ± 0.17 (45 min) and 0.90 ± 0.21 (60 min). Each point represents the mean and vertical lines indicate s.e.mean.

59.6 ± 6.4 μmol g^{-1} 40 min^{-1}. The enhanced NA overflow produced by perfusion with an anoxic perfusate containing no substrate was suppressed by 100 nM desipramine (Figure 2). At later times, however, NA overflows were higher in the desipramine series than in the control experiments. After 50 min anoxia NA overflows (pmol g^{-1} min^{-1}) were 22.5 ± 2.0 (control) and 27.3 ± 4.3 (desipramine), and at 60 min they were 15.7 ± 1.6 (control) and 22.4 ± 2.6 (desipramine). Desipramine was without effect on lactate production during substrate-free anoxia. Lactate overflows (μmol g^{-1} min^{-1}) were 2.57 ± 0.41 (10 min) and 1.02 ± 0.31 (20 min) in the absence of desipramine and 2.66 ± 0.54 (10 min) and 0.59 ± 0.35 (20 min) in the presence of desipramine. At 30 min mean overflows were < 0.25 μmol g^{-1} min^{-1} for both groups.

During anoxic, substrate-free perfusion, potassium concentrations in the effluent rose significantly by 0.1–0.2 mM (mean values 4.10 ± 0.03 and 4.22 ± 0.03 mM, $n = 15$) between 10 and 20 min of anoxia. No significant change in external Na^+ concentration was observed.

Restoration of perfusate to normal after a 10 min period of anoxic, calcium-free perfusion resulted in a transient rise in NA overflow to 2.40 ± 0.80 pmol g^{-1} min^{-1} (Figure 4a) in the first 2 min. Subsequent overflows were not greater than the pre-anoxic values.

In contrast LDH overflow rose from less than $0.1 \mathrm{u g^{-1} min^{-1}}$ at the end of the anoxic perfusion to a peak value of more than $30 \mathrm{u g^{-1} min^{-1}}$ following re-introduction of calcium and oxygen. After 20 and 30 min anoxia, NA overflows had already risen and on restoration of perfusate to normal there was a return of these elevated levels to pre-anoxic values despite the marked myocyte damage (LDH release) produced (Figure 4b, c).

The effect of repetitive stimulation of the left cervico-thoracic sympathetic ganglion (5 pulses s^{-1}, 0.8 mA) on NA overflow and heart rate increase for hearts perfused with oxygenated buffer containing glucose and pyruvate is shown in Figure 5. There was no significant decline in nerve stimulation-induced NA overflow or heart rate increase over the period tested. In the absence of substrate and oxygen there was a significant fall in NA overflow by 10 min; in the absence of substrate, but with oxygen, a significant decline in NA overflow had occurred by 30 min (Figure 6). In the presence of glucose, however, anoxia was without effect on stimulation-induced NA release which was maintained over the period tested (Figure 6).

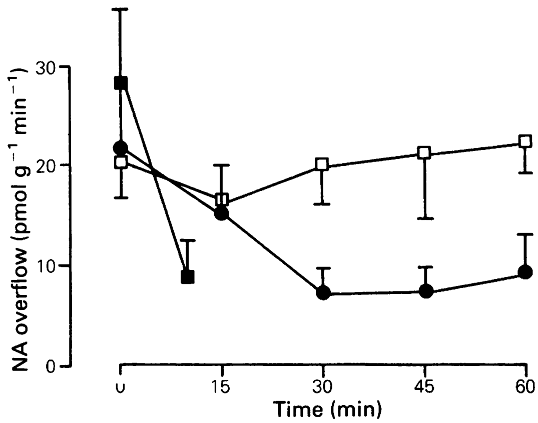

Figure 6 Noradrenaline (NA) overflow (pmol g^{-1} min^{-1}) produced by repetitive stimulation of the left cervico-thoracic ganglion at 5 pulses s^{-1}, 0.8 mA for 30 s. Perfusion was with anoxic perfusate ($P_{O_2} < 1$ mmHg) containing no substrate (■) ($n = 6$), anoxic perfusate containing 11 mM glucose (□) ($n = 6$) or normoxic ($P_{O_2} > 500$ mmHg) perfusate containing no substrate (●) ($n = 6$). In all experiments the initial stimulation was performed during perfusion with normoxic perfusate containing 5.5 mM glucose and 1.8 mM pyruvate. Perfusate was then changed as indicated. Pre-stimulation overflows (pmol g^{-1} min^{-1}) were 2.09 ± 0.89 (control), 2.37 ± 1.02 (15 min), 2.23 ± 0.84 (30 min), 2.40 ± 0.92 (45 min) and 2.77 ± 1.00 (60 min) in the anoxia with glucose series; 0.70 ± 0.07 (control), 0.83 ± 0.34 (15 min), 1.21 ± 0.50 (30 min), 0.62 ± 0.36 (45 min) and 0.57 ± 0.23 (60 min) in the glucose-free, normoxia series; 0.80 ± 0.16 (control) and 1.21 ± 0.11 (10 min) in the glucose-free, anoxia series. Each point represents the mean and vertical lines indicate s.e.mean.

Discussion

The experiments described demonstrate that the energy status of the sympathetic nerve terminal is the major determinant of the occurrence of nerve-impulse independent (non-exocytotic) noradrenaline release during myocardial ischaemia.

It is not possible to obtain information relating directly to the metabolic status of the sympathetic nerve terminals within the heart. However, during substrate-free anoxia the hearts rapidly become quiescent so that differences in energy utilization between neurones and myoctyes, as a consequence of mechanical activity, will be reduced. In addition, the major source of adenosine 5'-triphosphate (ATP) generation will be by anaerobic glycolysis of endogenous glycogen with the production of lactate which, in the absence of oxygen, cannot be utilized and can thus serve as an index of ATP generation. Under these conditions, over 80% of total lactate overflow had occurred by 15 min and, interestingly, it was at 15 min that anoxic release of NA became apparent. Provision of glucose almost completely suppressed NA overflow and allowed glycolytic activity to continue throughout the period studied. However, differences between myocyte and nerve terminal metabolism were also evident from the observation that, although pre-perfusion with a normoxic perfusate containing no substrate shortened the latent period before anoxic NA overflow, there was only a minor and non-significant reduction in anoxic lactate overflow. This implies that myocytes are better able to conserve glycogen during perfusion with normoxic, but substrate-free, perfusate than are the nerve terminals, probably by utilization of non-glucose endogenous substrate. Lactate overflow during anoxia should therefore be seen as a guide to events in the nerve terminal rather than as providing precise information of nerve terminal metabolism. Although following substrate depletion anoxic NA release was apparent earlier, the peak NA overflow achieved was not increased. This can be interpreted as indicating that although NA efflux occurs when ATP levels have fallen below a critical level the rate of efflux is then determined by other factors. The effects of calcium-free perfusion may also be partly due to changes in ATP generating capacity, since total lactate overflow was lower under these conditions, although the tendency to a higher peak NA overflow during calcium-free perfusion may indicate an additional mechanism.

In contrast to anoxia, low flow ischaemia, even in the absence of added substrate, did not lead to enhanced noradrenaline overflow in comparison to pre-ischaemic values. This can be explained by the much greater efficiency of oxidative metabolism in ATP generation in comparison to anaerobic glycolysis. Although global 90% ischaemia, following pre-ischaemic loading with [^{3}H]-noradrenaline, leads to an increased ^{3}H concentration in the venous effluent (Carlsson *et al.*, 1986) calculation of the data as overflow g^{-1} min^{-1} showed no increase above pre-ischaemic levels at 10 and 20 min, despite the use of lactate as the only exogenous substrate during ischaemia. After 60 min ischaemia, however, ^{3}H overflow g^{-1} min^{-1} was approximately twice the pre-ischaemic value (calculated from Table 1, Carlsson *et al.*, 1986). The initial fall in endogenous NA overflow during ischaemia can be partly explained by neuronal re-uptake of NA (Dart & Riemersma, 1985). Enhanced noradrenaline overflow does become apparent when substrate-free ischaemia is produced after a period of substrate depletion. It should, however, be noted that the peak NA overflow occurring under these conditions is several-fold lower than the peak overflow found with anoxia. This may be due to the much higher extracellular NA concentration found during ischaemia, reducing the intracellular/extracellular NA gradient and thereby limiting the rate of NA efflux from the neurones. Despite the striking difference in NA overflow between substrate-free and substrate-depleted ischaemia, there were no differences in lactate overflow, again implying that glycogen comprises the major energy reserve of the nerve terminals, whereas myocytes, in the presence of oxygen, are able to utilize alternative substrate reserves. The resistance of the nerve terminals to ischaemia, and the fact that the flow used is far less than the normal *in vivo* resting coronary flow of 3.8 ml g^{-1} min^{-1} (Wicker & Tarazi, 1982), implies that *in vivo* such nerve-impulse independent release will be restricted to regions of profound ischaemia, perhaps leading to heterogeneous stimulation of the ischaemic myocardium. NADH surface fluorescence data from ischaemic rat hearts have suggested that localized areas of severe ischaemia could exist (Steenberger *et al.*, 1977). Nerve-impulse independent noradrenaline release during anoxia and ischaemia was suppressed by desipramine, in keeping with the hypothesis that such release is due to carrier-mediated efflux (Schömig *et al.*, 1984). Desipramine did not alter the production of lactate during anoxia and is thus unlikely to act by increasing nerve terminal ATP generation. In addition, severity of ischaemia, as indicated by lactate production, was also unchanged by desipramine. Changes in myocardial contractility were not monitored but the hearts rapidly became quiescent and it is unlikely that, even if present, changes in the energy balance of the myocyte could have influenced the energy balance of the nerve terminal. Such a mechanism is conceivable *in vivo* if desipramine were associated with a reduced level of sympathetic activity, leading to less ATP utilization by the nerve terminal, but such a mechanism could not operate in the experiments described here. Even in the presence of desipramine, however, NA overflow was not completely suppressed and this desipramine-resistant release became more prominent at later stages of anoxia. The mechanism of this release has not been elucidated. Interpretation of NA overflow at later stages of anoxia was complicated by the inevitable depletion of NA which occurs following high early overflows and peak NA overflow may be a better indicator of NA efflux under different conditions.

Restoration of oxygen and substrate after a period of anoxia led to a fall in elevated NA overflow, even when combined with re-introduction of calcium. Re-introduction of calcium and oxygen did, however, lead to a massive release of the intracellular enzyme LDH ('calcium/oxygen paradox', Hearse *et al.*, 1978). This suggests that at least as far as non-exocytotic release is concerned, the processes are rapidly reversible as soon as the capacity for ATP generation is restored. There was also a marked difference between the myocyte and nerve terminal in response to the calcium/oxygen paradox.

It has been previously found that neurally mediated (exocytotic) NA release in the rat heart was depressed early during the course of severe ischaemia (Dart *et al.*, 1984). The experiments described here suggest that this is largely due to a failure of energy production by the nerve terminal since in these experiments other consequences of severe ischaemia (e.g. acidosis, hyperkalemia, metabolite accumulation) would not pertain, as the studies were all performed at relatively high flow rates. Glycolytic activity from exogenously supplied glucose is capable of supporting this release even in the absence of supplied oxygen.

In conclusion, our results show that nerve-impulse independent noradrenaline release can be expected to occur during severe myocardial ischaemia and is not dependent on the process of reperfusion. Desipramine suppresses this release and this may contribute to the anti-arrhythmic effect of desipramine and related drugs (Bril & Rochette, 1985; Daugherty *et al.*, 1986; Riemersma *et al.*, 1986). The metabolic status of the nerve terminal is the major determinant of whether nerve-impulse independent or dependent NA release will occur.

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